MUSCARINIC ACETYLCHOLINE RECEPTOR BINDING AGENTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 15/939,748, filed Mar. 29, 2018, pending, which is a divisional of U.S. patent application Ser. No. 14/765,824, filed Aug. 4, 2015, which is a national phase entry under 35 U.S.C. § 371 of International Patent Application PCT/EP2014/052265, filed Feb. 5, 2014, designating the United States of America and published in English as International Patent Publication WO 2014/122183 A1 on Aug. 14, 2014, which claims the benefit under Article 8 of the Patent Cooperation Treaty and under 35 U.S.C. § 119(e) to United States Provisional Patent Application Serial Nos. 61/761,136, filed Feb. 5, 2013, and 61/961,058 filed Oct. 3, 2013, the disclosure of each of which is hereby incorporated herein in its entirety by this reference.

STATEMENT ACCORDING TO 37 C.F.R. § 1.821(C) OR (E)—SEQUENCE LISTING SUBMITTED AS PDF FILE WITH A REQUEST TO TRANSFER CRF FROM PARENT APPLICATION

[0002] Pursuant to 37 C.F.R. § 1.821(c) or (e), files containing a TXT version and a PDF version of the Sequence Listing have been submitted concomitant with this application, the contents of which are hereby incorporated by reference.

TECHNICAL FIELD

[0003] Many transmembrane receptors such as G protein-coupled receptors (GPCRs) exist in many interconvertible three-dimensional conformations depending on their activity or ligand-binding state. Agents that specifically bind to a transmembrane receptor in a conformationally specific way can be used to induce a conformational change in the transmembrane receptor. Such agents have therapeutic applications and can be used in X-ray crystallography studies of the transmembrane receptor. Such agents can also be used to improve drug discovery via compound screening and/or structure based drug design.

BACKGROUND

[0004] Muscarinic acetylcholine receptors (M1-M5) are members of the G protein coupled receptor (GPCR) family that regulate the activity of a diverse array of central and peripheral functions in the human body, including the parasympathetic actions of acetylcholine (Wess et al., 2007). The M2 muscarinic receptor subtype plays a key role in modulating cardiac function and many important central processes such as cognition and pain perception (Wess et al., 2007). As it was among the first GPCRs to be purified (Peterson et al., 1984) and cloned (Kubo et al., 1986), the M2 receptor has long served as a model system in GPCR biology and pharmacology. Muscarinic receptors have attracted particular interest due to their ability to bind small molecule allosteric modulators (Mohr et al., 2003). Since allosteric sites can comprise receptor regions that are less conserved in sequence and structure than the orthosteric binding site, some ligands binding to allosteric sites in muscarinic receptors show substantial subtype selectivity (Digby et al., 2010; Keov et al., 2011). Such agents hold great promise for the development of novel muscarinic drugs for the treatment of various clinical conditions including diseases of the central nervous system and metabolic disorders. Though crystal structures were recently obtained for inactive states of the M2 and M3 muscarinic receptors (Haga et al., 2012; Kruse et al., 2012), experimental data regarding the structural basis for muscarinic receptor activation and allosteric modulation by drug-like molecules has not been reported. Such information could greatly facilitate the development of novel agents with increased potency and selectivity.

[0005] The binding of an activating ligand (agonist) to the extracellular side of a GPCR results in conformational changes that enable the receptor to activate heterotrimeric G proteins. Despite the importance of this process, only the β-adrenergic receptor and rhodopsin have been crystallized and their structures solved in agonist-bound active-state conformations (Choe et al., 2011; Rasmussen et al., 2011a; Rasmussen et al., 2011b; Deupi et al., 2012; Scheerer et al., 2008). Crystallization of agonist-bound active-state GPCRs has been extremely challenging due to their inherent conformational flexibility. Fluorescence and NMR experiments have shown that the conformational stabilization of the agonist-bound active-state conformation requires that the receptor must form a complex with an agonist and its G protein, or some other binding protein that stabilizes the active conformation (Yao et al., 2009, Nygaard et al., 2013).

[0006] The development of new straightforward tools for structural and pharmacological analysis of GPCR drug targets is therefore needed.

BRIEF SUMMARY

[0007] In a first aspect, the disclosure relates to a conformation-selective binding agent that is directed against and/or capable of specifically binding to a GPCR of the muscarinic acetylcholine receptor family. In a preferred embodiment, the above-described conformation-selective binding agent is directed against and/or is capable of specifically binding to muscarinic receptor M2 (M2R). It will be appreciated that M2R can be of any origin, preferably from mammalian origin, in particular from human origin.

[0008] The disclosure particularly envisages that the conformation-selective binding agent is capable of stabilizing M2R in a functional conformation, such as an active conformation, an inactive conformation, a basal conformation or any other functional conformation. Preferably, the conformation-selective binding agent is selective for an active conformation of the receptor.

[0009] In more specific embodiments, the above-described binding agent binds a conformational epitope of the receptor. In a preferred embodiment, the binding agent binds to an extracellular conformational epitope of the receptor. In another preferred embodiment, the binding agent binds to an intracellular conformational epitope of the receptor. A particular embodiment envisaged in the disclosure is that the above-described binding agent occupies the G protein binding site of the receptor. In one specific embodiment, the above-described binding agent is a G protein mimetic.

[0010] According to a preferred embodiment, the above-described binding agent comprises an amino acid sequence that comprises four framework regions (FR1 to FR4) and three complementarity-determining regions (CDR1 to CDR3), or any suitable fragment thereof. Preferably, the